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PREPRINT

Radiation Damage and Its Influence on Source Requirements for High Resolution X-Ray Holography

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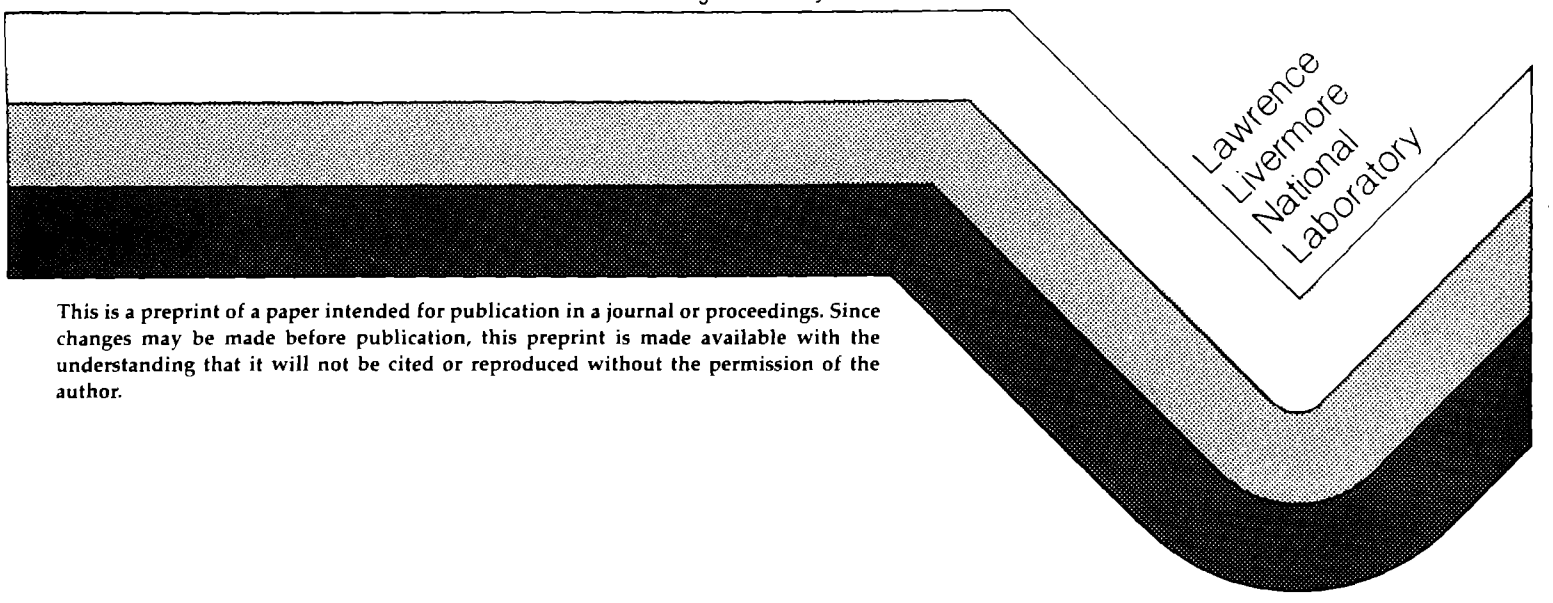

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Radiation Damage and its Influence on Source Requirements for High Resolution X-Ray Holography

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Summary

This paper outlines the talk given at the conference on X-ray Microimaging for the Life Sciences held May 24-26, 1989 in Berkeley, California. Much of the material is described in greater detail in ref. 1.

Soft x-ray holography offers the possibility of obtaining high resolution, 3-D images of living cells and organelles therein. To achieve a specified resolution, a certain number of photons must be scattered by the smallest features of interest within the sample. This requires a certain irradiating fluence, the magnitude of which depends on the wavelength of the x rays and the scattering cross-sections of the features. Unfortunately, irradiation of the sample will be accompanied by the absorption of x rays. If the dose is large, the sample will be damaged, possibly compromising the quality of the image.

A theoretical study of the scattering and absorption of x rays during the creation of a hologram is described. Using a new prescription for scattering by condensed biological materials (e.g. protein and/or DNA) within the aqueous environment of a cell, we estimate the irradiating fluence required for a certain resolution and the associated sample dose. The relative merits of different x-ray wavelengths are discussed. A wavelength of about 44Å, just outside the "water window" (23.2 – 43.7Å), appears to be optimal in that the required fluence and dose are minimized, while reasonable x-ray penetrability is maintained. Estimates are given for the minimum source energy required and the maximum duration of an exposure to capture an image before blurring due heat induce motion. The use of colloidal gold tagging can enhance image contrast and reduce the required irradiating fluence and sample damage.

A multi-disciplinary group has been studying the feasibility of producing high resolution 3-D images of living cells using soft x-rays

- **Physics:** R. London, D. Matthews, M. Rosen, A. Szöke, and J. Trebes
- **Biology:** J. Gray, D. Peters, and D. Pinkel
- **Elec. Engineering:** J. Brase and T. Yorkey

Activities of holography study group (and talks at microimaging meeting)

- Define candidate biological objects (talk by Gray)
- study x-ray interactions, damage, and source requirements (this talk)
- design x-ray holography system
- develop simulation and reconstruction codes (talk by Brase)
- field analog holography experiments using visible light
- develop appropriate x-ray lasers (talk by Matthews)

The main part of this paper concerns x-ray interactions with the sample

- scattering is essential to make a hologram.
- absorption is detrimental, it leads to damage and limits sample thickness.
- previous work has been done by Solem et al[2] and Howells and Jacobsen[3,4].

• we reconsider interaction properties of a wet biological sample and explore optimal x-ray wavelength considering three criteria:
  1. minimize the required x-ray fluence
  2. minimize the absorbed dose and subsequent damage
  3. maximize the penetration length (i. e. sample thickness)
• we study limits on exposure time due to heat buildup, explosive motion, and natural motions of living samples.
• we discuss implications for x-ray source.

The following formulas are used to study the x-ray interactions

• define a resolution element as smallest resolvable feature
• assume a certain number of photons (N_s) must be scattered by each resolution element, as determined by signal/noise and detector efficiency
• required fluence: $F = N_s h\nu / \sigma_s$ [units: erg / cm^2] ,
where $\sigma_s \equiv$ cross section of the resolution elements [units: cm^2]
• absorbed dose: $D = F \kappa_a$ [units: erg / g, Mrad = 10^8 erg / g]
where $\kappa_a \equiv$ absorption opacity [units: cm^2 / g]
• e-folding penetration depth: $L = 1/(\kappa_a \rho)$ [units: μm]

We assume that the smallest resolvable features can be approximated as spheres (Figure 1.)

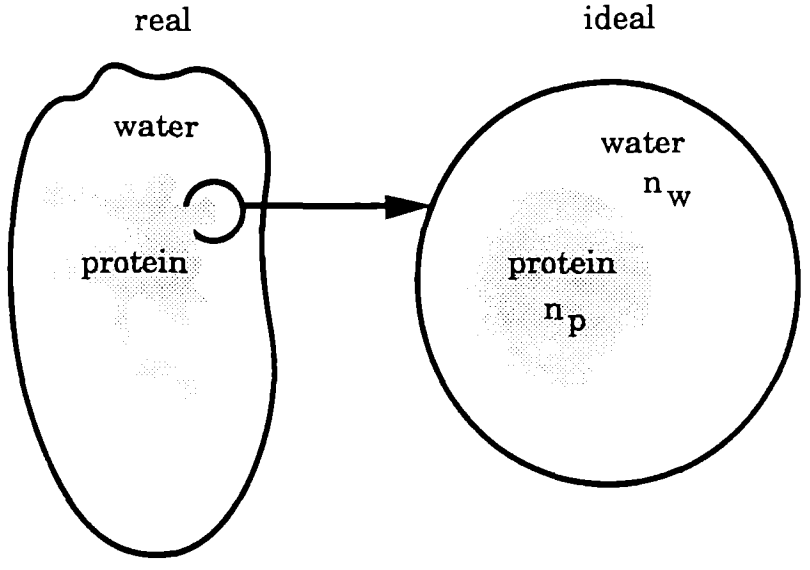

Figure 1. Spherical model for resolution elements.

We take the scattering cross section from the Rayleigh-Gans formula[5]

$$\sigma_s = \frac{\pi^2 d^4}{2\lambda^2} |n_p - n_w|^2$$

where $n_{p,w}$ are the complex indices of refraction of protein and water.
• valid for small phase shifts (equivalent to the Born approx.)

$$\text{let } n = 1 - \delta - i\beta \Rightarrow \sigma_s \propto (\delta_p - \delta_w)^2 + (\beta_p - \beta_w)^2$$

• optical constants (δ and β) are calculated from atomic scattering factors given in Henke[6] tables.
• both the real and imaginary parts of the index are important
• the <u>difference</u> between protein and water is important.

standard parameters for examples

- protein composition = $H_{49}\ C_{33}\ N_9\ O_9\ S_1$; density = 1.35 g / cm^3
- diameter of spherical resolution element: d = 300 Å.
- minimum number of scattered photons from each resolution element: $N_S = 10^3$.

We illustrate the wavelength dependence of the scattering cross section, the fluence and the dose in Figures 2, 3, and 4.

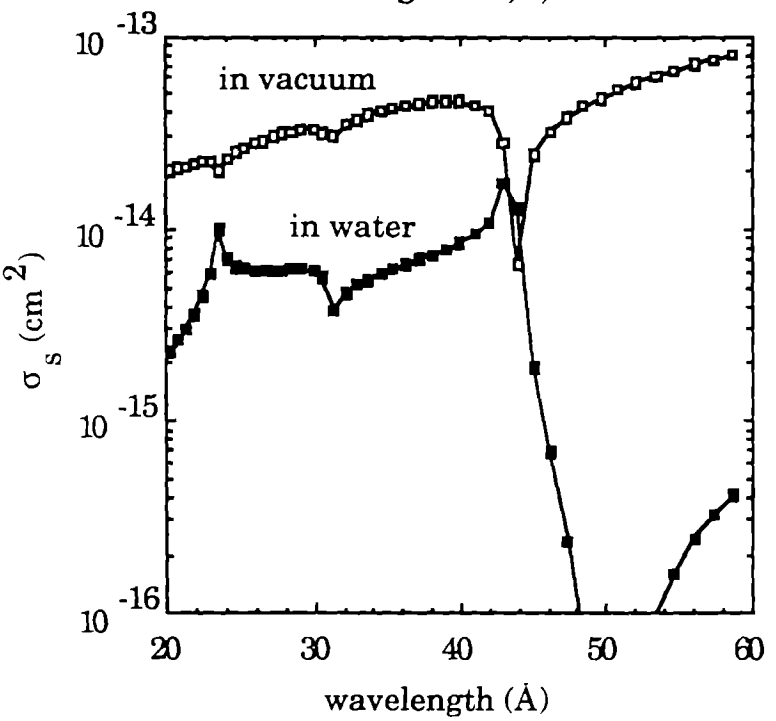

Figure 2. Scattering cross section for a 300 Å protein sphere in vacuum and in water

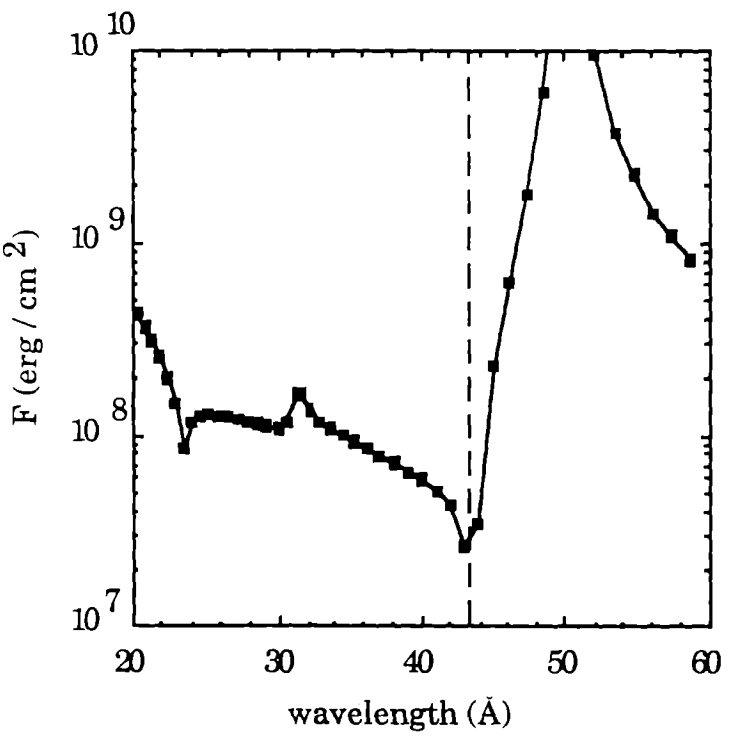

Figure 3. Fluence for a 300 Å protein sphere in water to scatter 1000 photons. The 43.7 Å C K-edge is indicated.

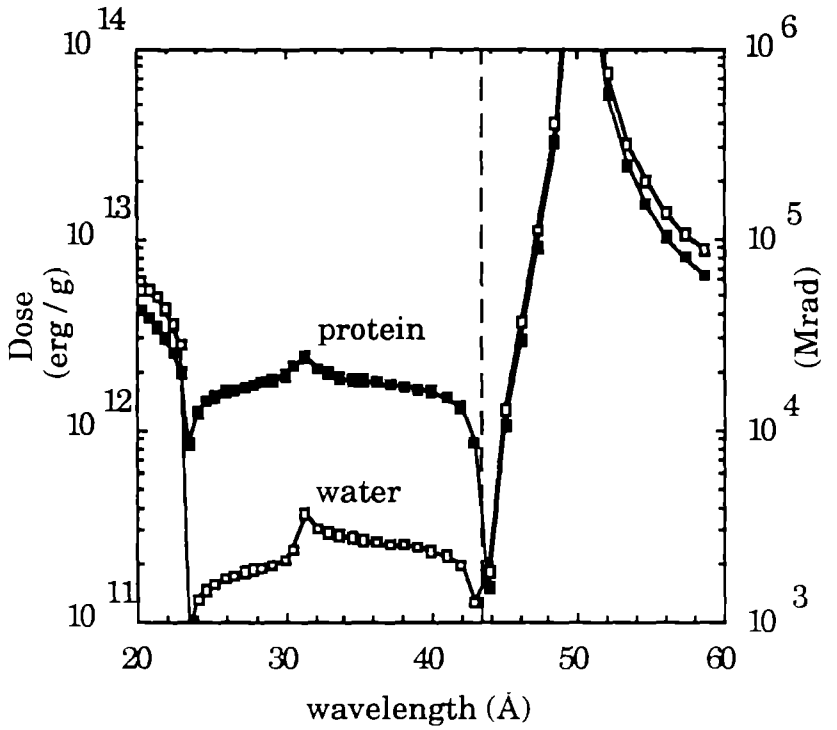

Figure 4. Dose to the protein and water for a 300 Å protein sphere in water to scatter 1000 photons.

We compare the estimated dose with several characteristic doses

process	dose (erg / g)
kill a hardy bacterium	10^8
chemical damage	$10^9 - 10^{11}$ (??)
boil water (ΔT = 100K)	4×10^9
x-ray holography at 44Å	2×10^{11} (d/300Å)$^{-4}$

Results for choice of wavelength, and resulting fluence and dose

- we suggest an optimal wavelength ≈ 44 Å for protein in water to minimize fluence and dose.
- the advantage of doing holography in the water window (λ = 23.3 to 43.7Å) appears to be a fallacy.
- for resolution = 300Å at λ = 44Å, we estimate:
  fluence ≈ 3×10^7 erg / cm^2
  dose ≈ 2×10^{11} erg / g
  penetration depths ≈ 2μm (in both water and protein)
- fluence and dose scale as d^{-4}.
- fluence and dose are higher than Howells[3] and Jacobsen[4];

main difference is that they assume an ellipsoidal resolution element rather than a spherical one.

Gold tagging to enhance contrast in x-ray imaging

- colloidal gold tagging has been developed for 15 years for electron microscopy.
- marking of specific sites can be accomplished using antibodies attached to gold particles 50 Å to 1500Å in diameter.
- for x-ray holography, the required fluence and dose are reduced by a factor of 60 using 300Å gold tags (see Fig. 5 for fluence).

- the optimal wavelength is still > 43.7 Å, with a broader range acceptable range.

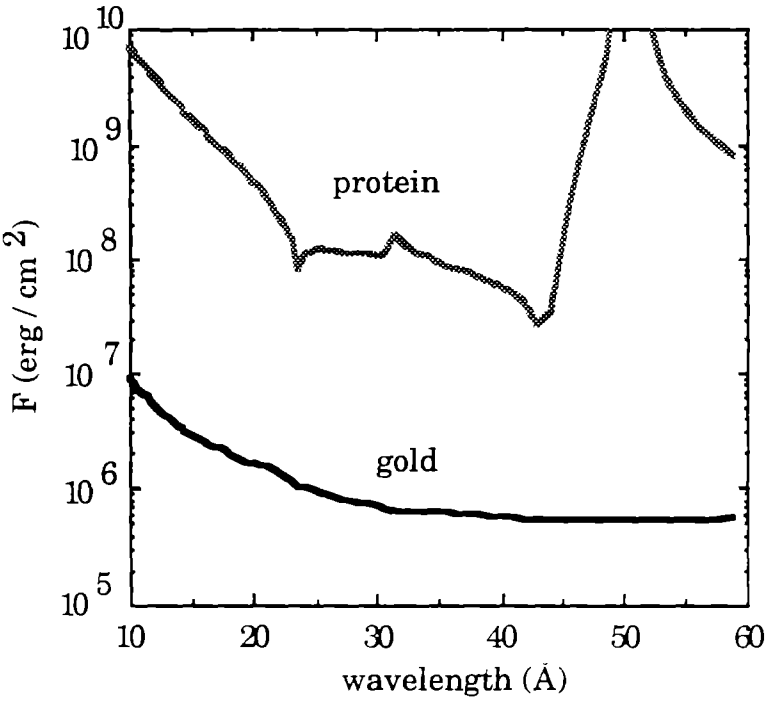

Figure 5. Fluence to scatter 1000 photons by 300 Å spheres of protein and gold

We now consider the limitations placed on the duration of the x-ray exposure by studying the scaling of several characteristic timescales

process	timescale (s)
natural	
biological dynamics	(10^{-3} ?)
brownian motion	$2\times10^{-3}\ d^2\ R_b$
Exposure duration	
conductive cooling	$6\times10^{-2}\ D\ R_c^2$
hydro - expansion	$3\times10^{-11}\ D^{-1/2}\ d$ (ref. 2)

The symbols in the preceding tables are defined as: d is resolution, scaled to 300Å; D is dose, scaled to 10^{11} erg / g; R_b is the radius of the object undergoing brownian motion, scaled to 1 μm; and R_c is the size of the cooled boundary of the sample where the heat is removed, scaled to 10 μm.

Several conclusions are drawn based on the dose - timescale considerations

- gold tagged samples may be imaged at low dose (~ 3×10^9) for which hydro motion is probably not an issue.
- natural samples require high doses (~ 2×10^{11} erg / g) for which there are two possible modes of imaging:
  long pulse ($t > 10^{-2}$ s), in which conduction keeps the sample cool.
  ultra short pulse ($t < 3\times10^{-11}$s), in which image is captured before hydrodynamic blurring compromises the resolution, as suggested by Solem[2].
- natural processes at resolution scale may be take place in ~10^{-3} s (as indicated by brownian motion), requiring short exposures.

Conclusions

- A study of the scattering properties of biological materials in water implies that the water window (23.3Å - 43.7Å) may not be optimal for holography
- $\lambda \approx 44$ Å minimizes the fluence and dose for a protein sample in water, while maintaining reasonable penetrability, and is therefore suggested as optimal.
- x-ray lasers with 300 μJ of coherent energy in a 50 psec pulse should be sufficient to produce holograms with 300Å resolution of natural samples
- with gold tagging, a factor of 60 in source energy can be saved. The dose is also reduced, and the short-pulse length requirements are alleviated.
- **By virtue of their short pulse (~50 ps) x-ray lasers can avoid the damage problem and image fast biological processes with high (~300Å) resolution**

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